Coffee and herbal tea consumption is associated with lower liver stiffness in the general population: The Rotterdam study

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Abstract

Introduction Coffee and tea have been proposed to limit progression of liver fibrosis in established liver disease, but it is unknown if this is also true for subclinical fibrosis. We therefore aimed to evaluate whether coffee and tea consumption are associated with liver stiffness in the general population.

Methods The Rotterdam Study is an ongoing prospective population-based cohort. We included participants who underwent transient elastography, ultrasound and completed a food frequency questionnaire. Coffee and tea consumption were categorized into no, moderate (>0–3), or frequent (≥3) intake (cups/day), and tea further into green, black and herbal tea (no/any). Significant fibrosis was defined as liver stiffness measurements (LSM) ≥8.0kPa. We performed regression analyses relating coffee and tea intake with fibrosis, steatosis and log-transformed LSM and adjusted for energy, sugar and creamer intake, age, gender, BMI, steatosis/LSM, HOMA-IR, ALT, alcohol, smoking, soda, healthy diet index and physical activity.

Results We included 2424 participants (age 66.5±7.4; 43% male) of whom 5.2% had LSM ≥8.0kPa and 34.6% steatosis. Proportion of LSM ≥8.0kPa decreased with higher coffee consumption (7.8%, 6.9% and 4.1% for no, moderate and frequent respectively; P_{trend}=0.006). This inverse association was confirmed in multivariable regression (OR_{mod} 0.75, 95%CI 0.33–1.67; OR_{freq} 0.39, 95%CI 0.18–0.86; *P*=0.005). Amongst tea consumers, only herbal tea consumers (36.3%) had lower log-transformed LSM after adjustment (Beta-0.05, 95%CI-0.08; –0.02, *P*=0.001). Subtypes of tea were associated with steatosis in univariate but not multivariable analysis.

Conclusion In the general population, frequent coffee and herbal tea consumption were inversely related with liver stiffness but not steatosis. Longitudinal analyses, as well as studies validating and unravelling underlying mechanisms are needed.

Introduction

Chronic liver diseases constitute a major public health problem. Liver cirrhosis was the 12th cause of death worldwide and the sixth cause of life-years lost in the adult population in developed countries in 2010.[76,77] Chronic liver diseases are often silent for over 20 years until cirrhosis develops. Indeed, several studies have suggested that liver fibrosis may be present within unselected individuals. Using transient elastography (TE) as diagnostic tool for liver fibrosis, a prevalence of 6–7% was found in the general population[78,79] and even up to 17% in those high-risk populations with metabolic syndrome and type 2 diabetes.[11] Lifestyle is an important factor in the pathogenesis of many liver diseases, examples of which include alcohol abuse in alcoholic liver disease and high caloric diet and inactivity in non-alcoholic fatty liver disease (NAFLD). At the same time, a healthy lifestyle, such as implementing a well-balanced diet or consumption of nutraceuticals, i.e. foods or nutrients with a health benefit, can prevent and even attenuate liver disease.[80]

Coffee and tea are the most consumed beverages worldwide and emerging as promising nutraceuticals for liver health.[81] Both beverages are part of well-rooted cultural traditions and also represent the second most traded commodity on world markets.[82] Consumption of these nutraceuticals has been associated with lower all-cause and cause-specific mortality, presumably through reducing risk of features of the metabolic syndrome.[83,84]

Coffee consumption was for the first time associated to liver health, that is lower liver enzymes, almost two decades ago.[85] Evidence supporting this protective effect of coffee on liver enzymes rapidly emerged henceforth.[86] Coffee consumption seemed to attenuate alcoholic liver disease[87] and studies in hepatitis C showed less severe fibrosis in coffee consumers.[88] Also in NAFLD patients, coffee consumption was inversely associated with fibrosis grade, but inconclusive concerning the relation to steatosis.[89-91] To date, only one study examined the relation between coffee and liver fibrosis in the general population, albeit using surrogate serum biomarker test as proxy for fibrosis, and found lower odds for fibrosis in frequent coffee consumers.[91]

The association between tea and liver health is less well established than that of coffee. All studies are either limited to Asian populations or include only serum transaminases as primary endpoint. In addition, results of these studies are inconclusive regarding the presumed health benefit of tea.[92-96]

To our knowledge, there are no studies examining whether coffee and tea consumption are associated with lower prevalence of steatosis and liver fibrosis, using reliable imaging techniques, in the general population. Hence, we conducted a cross-sectional analysis of individuals within a large prospective cohort study, who completed extensive dietary questionnaires, liver stiffness measurements (LSM), as proxy for fibrosis, and hepatic ultrasound (US) for the diagnosis of steatosis. Our aim was to determine whether coffee and tea consumption were associated with lower risk of liver fibrosis and steatosis in the general population.

Subjects and methods

Study population

This is a cross-sectional analysis of The Rotterdam Study, a large ongoing population-based cohort of participants aged 45 years and older living in a suburb of Rotterdam, The Netherlands. The rationale and design of this study have been described previously and a more detailed description of the design is added as *Supplementary Methods.*[97] For the purpose of our study, all participants visiting the research centre between January 2011 and September 2013 were included and underwent anthropometric assessment, abdominal US, TE and blood sampling. For all cohorts, this was the first hepatic examination in The Rotterdam Study. The Rotterdam Study has been approved by the institutional review board (Medical Ethics Committee) of the Erasmus MC University Medical Centre Rotterdam and by the review board of The Netherlands Ministry of Health, Welfare and Sports. Written informed consent was obtained from all participants.

Coffee and tea consumption

All participants completed an externally validated 389-item food frequency questionnaire (FFQ) developed for Dutch adults.[98,99] The questionnaire addressed type of food consumed over the last month, as well as frequency, portion size and preparation methods. Incomplete or unreliable FFQs, i.e. total energy intake less than 500 or more than 7500 kilocalories per day, were excluded. Questions regarding coffee and tea consumption included: "*How often did you drink coffee last month?*" and "*How often did you drink black / green / herbal tea (e.g. chamomile, red bush and nettle) last month?*" to which the possible answers were: "*1) not at all, 2)1 or 2–3 times, or 3)1; 2–3; 4–5 or 6–7 times a day*", and in case of daily consumption "*1–2; 3–4; 5–6; 7–8; 9–10 or 11 or more cups per day*". Coffee and tea consumption was thereafter categorized into no (0), moderate (>0–3) and frequent (≥3) consumption (in cups/day, one cup equals 150 grams). Tea consumption was further specified into herbal, green and black tea and subsequently dichotomized into no (0) and any (>0) consumption. Additionally, data on consumption of soda, alcohol, sugar and cream use in coffee or tea were obtained and used as covariates (in grams/day). Excessive alcohol consumption was defined as >14 units per week for women and >21 units per week for men (one unit equals 10 grams). Furthermore, to account for confounding by overall dietary quality, the Dutch Healthy Diet-index (DHDI) was added to multivariable analyses.[100]

Liver stiffness measurements and hepatic steatosis

LSM were performed using TE (Fibroscan®, EchoSens, Paris, France) by a single operator who had performed more than 1000 examinations before start of the study. Practical implementation of TE has been described previously.[78] The operator obtained 10 serial measurements of stiffness, using the M- or the XL-probe according to the manufacturer's instructions. Participants were excluded from our analyses if: 1) LSM did not meet the reliability criteria of Boursier et al., i.e. interquartile range (IQR)/ median LSM >0.30 with median LSM ≥7.1kPa;[101] 2) no LSM was obtained after at least 10 shots (defined as failure) and; 3) intra-cardiac devices or physical disabilities prohibited the use of TE.
Clinically relevant fibrosis was defined as LSM ≥8.0kPa and clinically relevant cirrhosis as LSM ≥13.0kPa. At these cut-off levels, previous studies showed high positive predictive values for presence of liver fibrosis and cirrhosis, respectively.[79,102,103]
Abdominal US was carried out by a certified and experienced technician on Hitachi HI VISION 900. Images were stored digitally and re-evaluated by a single hepatologist with more than 10 years of experience in US (PT). Diagnosis of steatosis was determined dichotomously according to the protocol of Hamaguchi et al.,[104] as presence or absence of a hyper echogenic liver parenchyma.

Biochemistry

Fasting blood samples were collected just before US and TE imaging. Blood lipids, platelet count, glucose, alanine aminotransferase (ALT), aspartate aminotransferase, gamma-glutamyltransferase (GGT), alkaline phosphatase and total bilirubin were measured using automatic enzyme procedures (Roche Diagnostic GmbH, Mannheim, DE). Insulin, hepatitis B surface antigen and anti-hepatitis C virus were measured by an automatic immunoassay (Roche Diagnostic GmbH). Patients with viral hepatitis were excluded from the analyses.

Additional Covariates

Data concerning demographics, education level, medical history, physical activity, comorbid conditions, smoking behaviour and drug use were obtained during an extensive home interview by trained interviewers. Detailed information on medication use was obtained from automated linkage to pharmacies with which 98% of the participants were registered. Anthropometric measurements were performed by well-trained research assistants. Body mass index (BMI) was calculated as weight (kg)/ height (m²) and waist circumference (WC) in centimetres. Blood pressure measurements were taken as the average of two subsequent measurements on the same day in upright position. According to the Adult Treatment Panel III criteria,[105] metabolic syndrome was diagnosed if at least 3 of the follow-

ing 5 traits were present: 1) abdominal obesity, defined as WC >102 cm (40 inch) in men and >88 cm (35 inch) in women; 2) serum triglycerides ≥150 mg/dl (1.0 mmol/L) or drug treatment for elevated triglycerides; 3) serum high-density lipoprotein cholesterol (HDL-C) <40 mg/dl (1.0 mmol/L) in men and <50 mg/dl (1.3 mmol/L) in women or drug treatment for low HDL-C; 4) Blood pressure ≥130/85 mmHg or drug treatment for elevated Blood pressure; 5) fasting plasma glucose (FPG) ≥100 mg/dl (5.6 mmol/L) or drug treatment for elevated blood glucose. Homeostasis model assessment of insulin resistance (HOMA-IR) was used as proxy for insulin resistance and calculated by multiplying fasting glucose (mmol/dl) by fasting insulin (mU/L) divided by 22.5.[106]

Statistical analyses

Population characteristics were described using descriptive statistics. Continuous data were presented as mean ± standard deviation or median with interquartile range (IQR) according to the distribution of the variable. Chi-square test, Student's T-test or Wilcoxon Rank Sum test were used to assess significance of differences in distribution of categorical, normally distributed and not-normally distributed data, respectively. Univariate linear and logistic regression analyses were performed to examine the association between coffee, overall tea and its subtypes herbal, green and black tea consumption and LSM as continuous and dichotomous (LSM <8.0kPa vs. LSM ≥8.0kPa) variable. In all regression models, multivariable adjustments were made by including age, gender, energy intake, BMI, insulin resistance, steatosis, serum ALT, excessive alcohol intake, soda consumption, smoking status, cream use in coffee and sugar use in tea or coffee, DHDI, type of probe, physical activity and education level as potential covariates.[107] To evaluate whether there was interaction with gender, HOMA-IR, BMI and steatosis on the one hand and coffee and tea consumption on the other hand, we evaluated the interaction terms (e.g. coffee x gender) in the multivariable model. In case of $P<0.05$ stratified analyses were performed. Additionally, correlation and multicollinearity was tested between steatosis and liver stiffness, given their strong relation, i.e. simple steatosis is a well-known risk factor for fibrosis. Furthermore, predicted probabilities of having LSM ≥8.0kPa were calculated, using the fully adjusted multivariable logistic regression model with LSM ≥8.0kPa as dependent variable. Probabilities were graphically depicted for the different coffee categories and presence of steatosis, to give more insight in these possibly interrelated covariates. Data are expressed as mean (95%CI) percentage. Also, different cut-offs for significant fibrosis were tested as outcome variable in univariate logistic regression, to emphasize the robustness of our findings. Additionally, a cut-off of ≥6.2kPa was used, indicating significant fibrosis in participants with steatosis. This cut-off has been proposed to exhibit high sensitivity for staging F2 fibrosis in participants with NAFLD, recently.[108] Univariate and multivariable logistic regression analyses were carried out assessing the association between coffee, tea and the secondary outcome

of interest, hepatic steatosis. In addition to the previous mentioned potential covariates, LSM was added to the model as well.

Several sensitivity analyses were performed to test the robustness of our data. Firstly, subgroup analysis was done by stratifying participants who filled in FFQ data 5.8 years prior to liver imaging (RS III-2) and, participants who filled in FFQ simultaneously with liver imaging (RS I-5 & RS II-3). Because dietary data are known to be stable over time,[109] RS III-2 was included in the total study population. Secondly, to ensure that the study population is a general population with no known liver disease (even though participants with viral hepatitis were excluded based on serology and participants were asked to report liver comorbidities during home interviewing), a sensitivity analysis was performed, excluding abnormal levels of ALT (> 2x upper limit of normal, which is 80 U/L for men and 60 U/L for women). A *P*-value of <0.05 was considered statistically significant. All analyses were performed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA).

Results

Study population

The flowchart of the study population is depicted in *Figure 1*. Of the 3439 participants visiting the research centre, participant failure of LSM was reported in 162 (4.8%) and unreliable results in 139 (4.2%) participants. In addition, 34 (1.1%) participants with unreliable FFQs and 566 (16.4%) with incomplete FFQs were excluded. Hence, the total study population was n=2424. Population characteristics are presented in *Table 1*. Fifty-seven percent of the participants were women, mean age was 66.5±7.4 years, mean BMI 27.2±4.0 kg/m² and median LSM was 4.7kPa (3.8–5.8). A total of 125 (5.2%) participants had LSM ≥8.0kPa, suggesting presence of significant fibrosis.

Data on coffee and tea consumption

Dietary data are shown in *Table 1*. In total, 2258 participants (93.2%) consumed coffee with a mean consumption of 2.6±1.7 cups/day. Frequent coffee consumers were proportionally more overweight or obese. Furthermore, it was associated with younger age, male gender, Caucasian ethnicity, higher education level, more current or past smoking, lower prevalence of lipid disorders and type 2 diabetes mellitus *(Supplementary Table 1)*. No difference was observed in serum ALT and HOMA-IR. Tea was consumed by 2052 (84.7%) participants, with a median of 1.2 (0.4–2.7) cups/day. Frequent tea consumption was associated with female gender, less excessive alcohol use, less smoking, lower BMI and less features of the metabolic syndrome *(Supplementary Table 1)*. Amongst subtypes of tea,

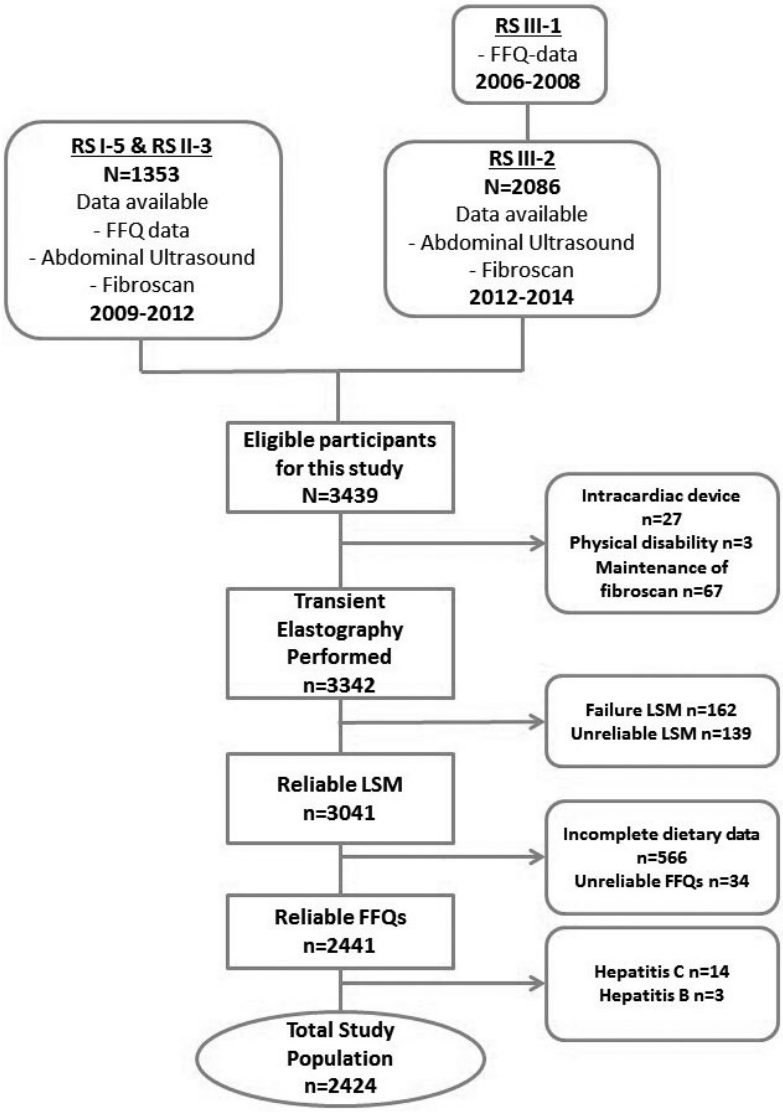


Figure 1: Flowchart of the study
Legend: RS= Rotterdam Study, I-III= number of cohort, 1–3 and 5= times cohort visited.

black tea was most commonly consumed (64.2%) followed by herbal (36.3%) and green tea (26.1%). Only 32 (1.3%) participants reported neither coffee nor tea consumption.

Table 1: Study Characteristics

	Total N=2424	LSM <8.0kPa N=2299	LSM ≥8.0kPa N=125	*P*-value
Demographics				
Age (years)	66.5±7.4	66.4±7.3	68.7±8.4	0.003
Female	56.6	57.7	35.2	<0.001
Caucasian	96.3	96.2	98.4	0.322
Low/Intermediate /High education	45.0/29.6 /25.4	45.2/29.4 /25.4	42.3/34.1 /23.6	0.531
Excessive alcohol use	15.9	15.9	16.0	1.000
Current/Past /Never smoker	11.3/52.9 /35.7	11.0/52.3 /36.6	16.8/63.9 /19.3	<0.001
Physical activity*	46 (19–85)	46 (19–85)	42 (18–85)	0.748
Physical examination				
BMI (kg/m^2)	27.2±4.0	27.1±3.9	28.8±5.3	0.001
-Normal	29.7	30.1	22.8	0.003
-Overweight	49.1	49.4	43.9	
-Obese	21.2	20.5	33.3	
WC (cm)				
-Men	99.1±10.2	98.9±9.9	101.1±13.1	0.152
-Women	89.0±11.5	88.7±11.3	96.6±15.7	0.002
Biochemistry[¥]				
Aspartate transaminase (U/L)	24 (21–28)	24 (21–28)	27 (24–37)	<0.001
ALT (U/L)	18 (14–24)	18 (14–23)	22 (16–33)	<0.001
Bilirubin (µmol/L)	8 (6–11)	8 (6–11)	9 (6–12)	0.252
GGT (U/L)	24 (17–35)	23 (17–34)	38 (26–71)	<0.001
Platelets (*10^9/L)	267.8±63.5	268.7±63.6	251.7±59.7	0.004
HOMA-IR	2.5 (1.7–3.9)	2.5 (1.7–3.8)	3.7 (2.1–7.1)	<0.001
Comorbidities				
Metabolic Syndrome	44.5	43.7	59.3	0.001
-WC >88cm (♀) or >120cm (♂)	44.1	43.5	53.7	0.032
-Triglycerides >150mg/dL	38.2	38.0	41.6	0.450
-HDL-C <40mg/dL (♂) or 50mg/dL (♀)	33.6	33.3	40.0	0.144
-Blood pressure ≥130/85mmHg[¥]	79.0	78.5	88.0	0.020
-FPG >100mg/dL	44.6	43.3	68.0	<0.001
Diabetes Mellitus	10.5	9.2	33.6	<0.001
Hypertension[†]	64.7	63.8	81.5	<0.001
Liver Imaging				
LSM (kPa)	4.7 (3.8–5.8)	4.6 (3.8–5.6)	9.1 (8.6–10.4)	<0.001
Steatosis	34.6	33.2	60.0	<0.001

Table 1 (*continued*)

	Total N=2424	LSM <8.0kPa N=2299	LSM ≥8.0kPa N=125	*P*-value
Dietary Data				
Coffee consumption (cups/day)	2.6±1.7	2.6±1.6	2.2±1.7	0.019
-None	6.8	6.7	10.4	0.006
-Moderate (>0–3)	28.8	28.3	38.4	
-Frequent (≥3)	64.4	65.1	51.2	
Tea consumption (cups/day)	1.2 (0.4–2.7)	1.2 (0.4–2.7)	1.2 (0.2–2.3)	0.211
-None	15.3	15.1	20.0	0.256
-Moderate (>0–3)	56.4	56.4	56.0	
-Frequent (≥3)	28.3	28.5	24.0	
Tea subtypes (%)				
Black	64.2	64.4	61.6	0.528
Green	26.1	26.3	21.6	0.242
Herbal	36.3	36.8	27.2	0.035
Other				
Alcohol (units/day)	0.8 (0.1–1.8)	0.8 (0.1–1.8)	0.9 (0.1–2.1)	0.334
Soda (%)	47.7	47.5	51.2	0.462
Kilocalorie intake	2189.7±740.3	2189.1±735.5	2199.7±826.2	0.877
Cream use	46.0	45.8	50.4	0.357
Sugar use	25.5	24.9	37.6	0.002

Data is presented as mean (±SD), median (IQR) or percentage. *P*-value is based on T-test, Wilcoxon rank sum test, Chi-square test or Fisher's exact test. *Physical activity in metabolic equivalent task hours/week. ¥Biochemistry data was available for 2320 and †BP data for 2051 participants.

Coffee consumption and liver stiffness

Proportion of participants with LSM ≥8.0kPa decreased with increasing coffee consumption (7.8% in no, 6.9% in moderate and 4.1% in frequent coffee consumption groups, P_{trend}=0.006). Using no coffee consumption as reference, univariate logistic regression showed an inverse association between coffee consumption and LSM ≥8.0kPa (OR_{mod} 0.87, 95%CI 0.46–1.64; OR_{freq} 0.50, 95%CI 0.27–0.94; *P*=0.007). This association enhanced after adjusting for energy intake, age, gender, BMI, steatosis, HOMA-IR, ALT, excessive alcohol use, current or former smoking, soda, DHDI, physical activity, total tea consumption and cream and sugar use in coffee (OR_{mod} 0.75, 95%CI 0.33–1.67; OR_{freq} 0.39, 95%CI 0.18–0.86; *P*=0.005) as shown in *Table 2*. Additional adjustment for education level, and type of probe did not affect this association (data not shown). Coffee consumption was also inversely related to log-transformed LSM as shown in a stepwise multivariable linear regression model in *Table 2* (P_{trend}=0.001). Interactions between covariables and coffee consumption were tested in all regression models, but found to be not significant.

Table 2: Association between coffee, tea and LSM by 1) stepwise linear regression modelling using log-transformed LSM as dependent variable and 2) stepwise logistic regression modelling using LSM ≥ 8 kPa as dependent variable. Non-consumers were used as reference.

	log-transformed LSM			LSM ≥ 8 kPa		
	Beta	**95%CI**	***P*-value**	**OR**	**95%CI**	***P*-value**
Model 1*						
Coffee						
No	0 (ref)		<0.001	1 (ref)		0.003
Moderate (>0–3)	–0.003	–0.058; 0.051		0.84	0.44; 1.61	
Frequent (≥3)	–0.055	–0.106; –0.003		0.46	0.24; 0.87	
Herbal Tea						
No	0 (ref)		<0.001	1 (ref)		0.024
Any	–0.100	–0.128; –0.071		0.61	0.40; 0.94	
Green Tea						
No	0 (ref)		0.076	1 (ref)		0.274
Any	–0.028	–0.059; 0.003		0.81	0.56; 1.18	
Black Tea						
No	0 (ref)		0.014	1 (ref)		0.476
Any	–0.034	–0.061; –0.007		0.85	0.54; 1.34	
Total Tea						
No	0 (ref)		<0.001	1 (ref)		0.116
Moderate (>0–3)	–0.073	–0.110; –0.035		0.74	0.46; 1.20	
Frequent (≥3)	–0.106	–0.147; –0.064		0.56	0.32; 0.97	
Model 2¥						
Coffee						
No	0 (ref)		0.001	1 (ref)		0.012
Moderate (>0–3)	–0.016	–0.069; 0.036		0.86	0.44; 1.70	
Frequent (≥3)	–0.060	–0.110; –0.010		0.50	0.26; 0.97	
Herbal Tea						
No	0 (ref)		<0.001	1 (ref)		0.446
Any	–0.051	–0.079; –0.023		0.84	0.54; 1.31	
Green Tea						
No	0 (ref)		0.304	1 (ref)		0.638
Any	0.016	–0.014; 0.046		1.12	0.70; 1.80	
Black Tea						
No	0 (ref)		0.131	1 (ref)		0.652
Any	–0.020	–0.046; 0.006		0.92	0.62; 1.35	
Total Tea						
No	0 (ref)		0.205	1 (ref)		0.945
Moderate (>0–3)	–0.035	–0.071; 0.001		0.95	0.58; 1.55	
Frequent (≥3)	–0.032	–0.073; 0.009		0.91	0.51; 1.62	

Table 2.(*continued*)

	log-transformed LSM			LSM ≥ 8 kPa		
	Beta	**95%CI**	***P*-value**	**OR**	**95%CI**	***P*-value**
Model 3[†]						
Coffee						
No	0 (ref)		0.001	1 (ref)		0.005
Moderate (>0–3)	–0.026	–0.083; 0.032		0.75	0.33; 1.67	
Frequent (≥3)	–0.067	–0.121; –0.013		0.39	0.18; 0.86	
Herbal Tea						
No	0 (ref)		0.001	1 (ref)		0.289
Any	–0.051	–0.081; –0.022		0.76	0.45; 1.27	
Green Tea						
No	0 (ref)		0.146	1 (ref)		0.424
Any	0.023	–0.008; 0.055		1.24	0.73; 2.10	
Black Tea						
No	0 (ref)		0.400	1 (ref)		0.615
Any	–0.012	–0.039; 0.016		0.88	0.52; 1.47	
Total Tea						
No	0 (ref)		0.514	1 (ref)		0.932
Moderate (>0–3)	–0.023	–0.061; 0.015		1.01	0.58; 1.77	
Frequent (≥3)	–0.063	–0.061; 0.024		1.11	0.58; 2.13	

[*]Model 1: adjusted for tea[‡] or coffee and energy intake
[¥]Model 2: adjusted for tea[‡] or coffee, energy intake, BMI, gender and age.
[†]Model 3: adjusted for tea[‡] or coffee, energy intake, BMI, gender, age, steatosis, ALT, excessive alcohol intake, current or former smoking and HOMA-IR, soda consumption, cream and sugar use, DHDI and physical activity.
[‡]All regression models either contain total tea or tea subtypes as covariate.

Additionally, steatosis and log-transformed liver stiffness were significantly correlated (r=0.152, *P*<0.001) but there was no multicollinearity (VIF 1.33). Nevertheless, steatosis remains an important well-known risk factor for the development of fibrosis. We therefore graphically depicted the adjusted predicted probabilities of having LSM ≥8.0kPa for the different coffee categories and presence of steatosis in Figure 2. As expected, the probability of having LSM ≥8.0kPa is higher for participants with steatosis than for those without (*P*<0.001). Within both the no steatosis and steatosis groups, the probability of LSM ≥8.0kPa for frequent coffee consumption is lower compared to no consumption (within no steatosis: 2.5% in frequent vs. 4.4% in no coffee consumption; $P_{\text{freq vs.no}}$=0.218 and within steatosis: 6.9% in frequent vs. 13.1% in no coffee consumption; $P_{\text{freq vs.no}}$=0.036).

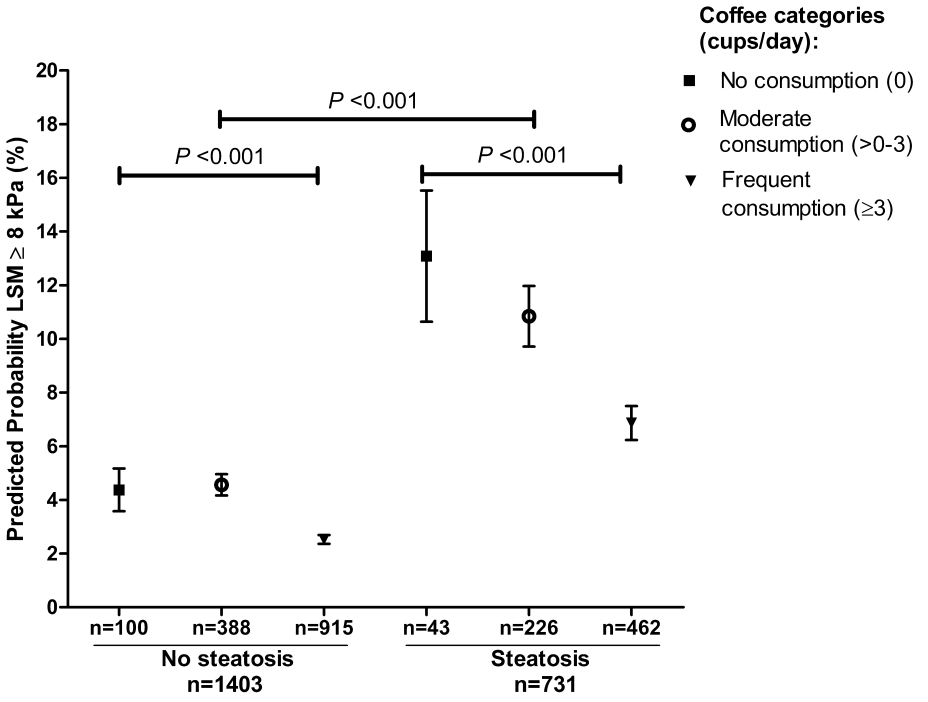


Figure 2: Predicted probabilities (%) of having LSM ≥8.0kPa for the different coffee categories amongst participants with and without steatosis

Y-axis: predicted probabilities of LSM ≥8.0kPa in percentages. Data are expressed as mean (95%CI) prediction. X-axis: different coffee consumption categories for participants with and without steatosis. Probabilities are adjusted for energy intake, age, gender, BMI, steatosis, HOMA-IR, ALT, excessive alcohol use, current or former smoking, soda, DHDI, physical activity, total tea consumption, cream and sugar use in coffee. The Ptrend is derived from the logistic regression analysis stratified for steatosis with the following results: in participants without steatosis: ORmod 1.19, 95%CI 0.35–4.06; ORfreq 0.47, 95%CI 0.14–1.57; P=0.025; Ptrend 0.017; and in participants with steatosis: ORmod 0.45, 95%CI 0.15–1.40; ORfreq 0.31, 95%CI 0.11–0.92; P=0.082; Ptrend 0.034. Interaction term was tested and found to be non-significant (Pinteraction=0.527 for steatosis x coffee). The comparison of predicted probability between the non-steatosis and steatosis group was based on the independent t-test (P<0.001).

Tea consumption and liver stiffness

Overall tea consumption was similar in participants with versus without LSM ≥8.0kPa *(Table 1).* Likewise, tea was not associated with log-transformed LSM in linear regression or with LSM ≥8.0Kpa in logistic regression when correcting for potential confounders *(Table 2).* Looking at subtypes of tea, only herbal tea was less frequently consumed by participants with LSM ≥8.0kPa compared to participants with normal LSM (*Table 1*), this finding remained when adjusted for energy intake and coffee consumption (OR 0.61, 95%CI 0.40–0.94; *P*=0.024). After further adjustment for lifestyle and metabolic traits herbal tea was not independently associated with LSM ≥8.0kPa, however, it remained

inversely related to log-transformed LSM values in a multivariable linear regression model as shown in *Table 2* (*P*=0.001). Interactions between covariables and herbal tea consumption were tested and not significant.

Tea, coffee consumption and steatosis

Steatosis was found in 34.6% of the study population and was higher amongst participants with LSM ≥8.0kPa (60% vs. 33.2%; *P*<0.001). Although proportion of steatosis was similar amongst coffee consumption groups (*P*=0.656), proportion differed significantly in tea consumers (42.7%, 35.4% en 28.6% for no, moderate and tea frequent consumers; *P*<0.001). Likewise, tea consumption was associated with steatosis in logistic regression analysis, in contrast to consumption of coffee. However, when correcting for potential confounders in a multivariable logistic regression model, this association was no longer significant, as shown in *Table 3*.

Table 3: Stepwise logistic regression models with steatosis as dependent variable using non-consumers as reference

	OR	95%CI	*P*-value
Model 1*			
Coffee			
No	1 (ref)		0.239
Moderate (>0–3)	1.32	0.92; 1.91	
Frequent (≥3)	1.17	0.82; 1.66	
Herbal Tea			
No	1 (ref)		0.039
Any	0.82	0.68; 0.99	
Green Tea			
No	1 (ref)		0.018
Any	0.78	0.63; 0.96	
Black Tea			
No	1 (ref)		0.006
Any	0.78	0.66; 0.93	
Total Tea			
No	1 (ref)		<0.001
Moderate (>0–3)	0.72	0.57; 0.92	
Frequent (≥3)	0.53	0.41; 0.69	
Model 2¥			
Coffee			
No	1 (ref)		0.186
Moderate (>0–3)	1.31	0.87; 1.98	

Table 3 (*continued*)

	OR	95%CI	*P*-value
Frequent (≥3)	1.09	0.74; 1.62	
Herbal Tea			
No (0)	1 (ref)		0.674
Any	0.96	0.77; 1.18	
Green Tea			
No	1 (ref)		0.297
Any	0.89	0.70; 1.11	
Black Tea			
No	1 (ref)		0.086
Any	0.84	0.69; 1.03	
Total Tea			
No	1 (ref)		0.064
Moderate (>0–3)	0.85	0.65; 1.10	
Frequent (≥3)	0.70	0.52; 0.95	
Model 3†			
Coffee			
No	1 (ref)		0.192
Moderate (>0–3)	1.38	0.88; 2.16	
Frequent (≥3)	1.15	0.75; 1.77	
Herbal Tea			
No	1 (ref)		0.779
Any	1.03	0.82; 1.29	
Green Tea			
No	1 (ref)		0.425
Any	0.91	0.71; 1.15	
Black Tea			
No	1 (ref)		0.169
Any	0.86	0.70; 1.06	
Total Tea			
No	1 (ref)		0.197
Moderate (>0–3)	0.86	0.65; 1.14	
Frequent (≥3)	0.74	0.54; 1.03	

* Model 1: adjusted for tea‡ or coffee and energy intake
¥ Model 2: adjusted for tea‡ or coffee, energy intake, BMI, gender and age.
† Model 3: adjusted for tea‡ or coffee, energy intake, BMI, gender, age, HOMA-IR, excessive alcohol intake, current or former smoking. Additional adjustment for LSM did not affect this association.
‡All regression models either contain total tea or tea subtypes as covariate.

Sensitivity analyses

In 1360 participants (56.1%), FFQs were completed 5.8 years prior to TE examination. As can be seen from this sensitivity analysis presented in *Supplementary Table 2,* results in both subgroups were consistent to that of the total study population, underlining the stable character of dietary data. Furthermore, 13 (0.5%) participants had LSM ≥13.0kPa, suggestive of cirrhosis. As expected, the association between coffee and LSM ≥13.0kPa was also found to be significant (OR_{mod} 0.35, 95%CI 0.10–1.26; OR_{freq} 0.08, 95%CI 0.02–0.35; *P*=0.004). The inverse, univariate relationship between different cut-offs of LSM and coffee consumption is graphically presented in *Figure 3*. Moreover, when applying a more stringent cut-off for significant fibrosis, i.e. LSM ≥6.2kPa, in participants with steatosis, similar results were found, albeit slightly attenuated and statistically not significant (OR_{mod} 0.62, 95%CI 0.28–1.38 and OR_{freq} 0.47, 95%CI 0.22–1.02; *P*=0.096). Lastly, exclusion of abnormal ALT levels from all analyses, to ensure results are generalizable for the general population without known liver disease, did not alter the results (data not shown).

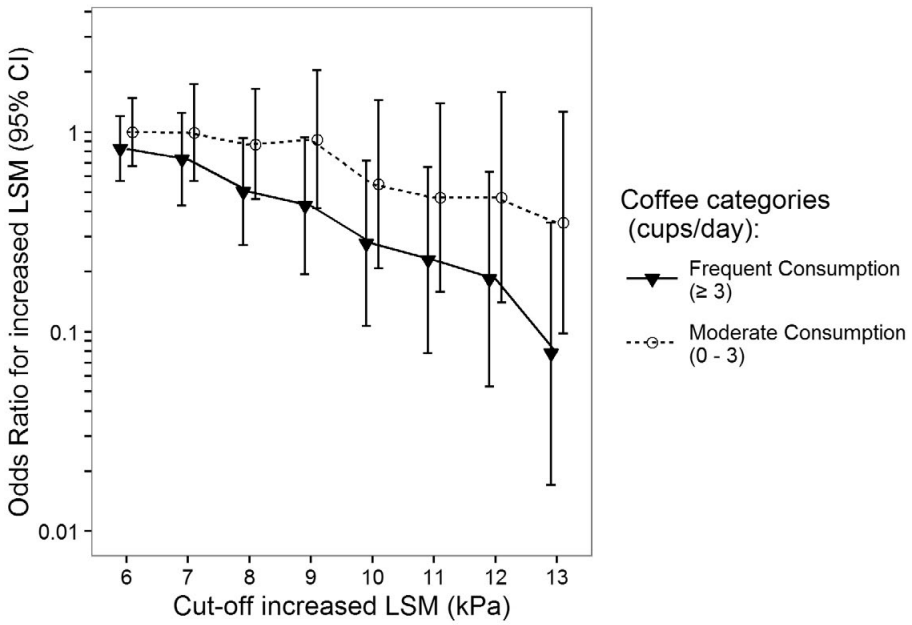


Figure 3: Estimated odds ratio for moderate and frequent coffee consumers versus no coffee consumers for a grid of different cut-off values of LSM

Results of the univariate logistic regression analyses of the effect of coffee consumption for the endpoint of LSM greater than a specific cut-point (yes/no). Y-axis: univariate OR (95%CI) on log-scale. X-axis: different cut-off values for LSM.

Discussion

To the best of our knowledge, this is the first study to determine whether coffee and tea consumption are associated with subclinical fibrosis and steatosis, using reliable imaging techniques, in the general population. This large population-based cohort study shows an inverse and independent association between coffee and herbal tea consumption and log-transformed LSM. Frequent coffee consumption was also independently associated with lower odds and probability of significant liver fibrosis (defined as LSM ≥8.0kPa) compared to non-consumers, independent of steatosis presence and a great number of metabolic and environmental traits.

A recent meta-analysis showed that, amongst liver disease patients, coffee consumers were less likely to develop liver fibrosis and cirrhosis than no-coffee consumers.[110] Yet, all studies included in this meta-analysis concerned specific, non-generalizable populations, i.e. patients with cirrhosis, other chronic liver disease or morbid obesity.[88,89,111] In addition, a study from a NASH consortium showed that coffee consumption was independently associated with lower odds (OR 0.64) of advanced histology-proven fibrosis in subjects with low insulin resistance.[90] Another study found that NASH patients with advanced fibrosis, i.e. fibrosis score 2–4, consumed less coffee than patients with fibrosis score 0–1.[112] But to date, a study of Zelber-Sagi et al. is the only one on the association between coffee and liver fibrosis in the general population, albeit using Fibrotest (i.e. a commercial biomarker test combining serum markers age and gender) rather than biopsy or TE, to assess fibrosis.[91] In line with our data, an association between prevalence of NAFLD and coffee consumption was not found. The authors found a lower proportion of significant fibrosis (defined as Fibrotest score ≥2, borderline F1-F2 included) in participants consuming 3 or more coffee cups/day (OR 0.49), which is also comparable to our data. However, the authors did not include a clear reference group, i.e. non-consumers, in their analyses and the association was only significant in univariate fashion. In contrast, we found that this association in fact enhanced after adjustment for several potential metabolic and environmental confounders. We hypothesize that this can be explained by the relatively unhealthy lifestyle of coffee consumers, as they were more often heavier drinkers, current or former smokers and obese.

Only few studies have assessed the effect of overall tea consumption on liver disease and these show inconsistent results.[92,111,113] Moreover, five large Asian studies specifically examined green tea consumption in relation to liver health,[93,94] of which three found a significant beneficial association between green tea and serum transaminases.[95,96,114] However, this association was only seen in individuals consuming more than 700ml green tea per day. We could not detect a beneficial effect of green tea in our study. This may partly be explained by our predominantly elderly Caucasian population, in which drinking green tea is not customary, as reflected in the low consumption rate. We speculate that

the beneficial effects of tea could be dose-dependent and might only exert an independent effect on liver health when consumed above a certain threshold. Furthermore, considering the favourable lifestyle traits in frequent tea consumers, a univariate association of (green) tea with liver stiffness and steatosis could be confounded by a healthy lifestyle in general. Interestingly, we did find herbal tea to be independently associated with lower liver stiffness. To the best of our knowledge, no other study has addressed this association. Although herbal tea encompasses a broad spectrum of different types of herbs, such as *Chamomile* and *Calicotome villosa*, and methods of preparation, a study of Carlsen et al.[115] stated that, despite the great diversity, 'herbal plants' are amongst the most antioxidant-rich food items and supplements worldwide and contribute the most to antioxidant capacity as assessed by ferric-reducing ability of plasma. We therefore hypothesize that the antioxidant capacity of herbal tea exceeds that of other teas, displaying a more pronounced effect on liver health. In addition, it has been previously demonstrated that the ferric-reducing ability of plasma is reduced in patients with steatosis and NASH.[12] However, future studies are needed to confirm this observation.

Mechanisms through which coffee and herbal tea promote liver health are unclear. Also, it is not known if these two beverage act through similar pathways. However, our data suggest that both have inhibitory properties for fibrogenesis and not steatogenesis. Both coffee and tea consist of more than 100 individual components, of which few, i.e. polyphenols and caffeine, are shared. Shim et al. showed that caffeine inhibits hepatic stellate cell proliferation in cirrhotic rat models and subsequently counteracted fibrogenesis.[116] Interestingly, caffeine in coffee had a greater effect on insulin sensitivity than caffeine in water alone. Moreover, another animal study found that conventional coffee, decaffeinated coffee and caffeine alone all reduced serum levels of ALT and fibrosis scores. But, only rats fed with conventional coffee showed significantly less histologic injury from repeated administration of toxics.[117]

Since caffeine alone does not seem to fully explain the beneficial effect of coffee, other components are proposed to contribute. Both coffee and tea contain a substantial amount of polyphenols, which have been suggested to promote hepatoprotective effects independent of caffeine.[118] Animal studies showed that coffee-polyphenols, chlorogenic acid and tocopherols, attenuate obesity-related lipid accumulation in the liver[119] and have antioxidant properties. Furthermore, experimental animal data suggest that tea-polyphenols attenuate fibrosis, oxidative stress and inflammation through pivotal inflammatory transcription factor[120] and inhibition of hepatic stellate cell activation.[121] In our population study, we could not explore the underlying mechanistic pathway responsible for the observed effects.

A major strength of this study is the inclusion of a large number of subjects from an unselected population, correcting for a great number of environmental and metabolic traits and, using well-validated diagnostic tools. Furthermore, we performed several sensitivity analyses, which should be interpreted in light of decreased power, showing consistency in

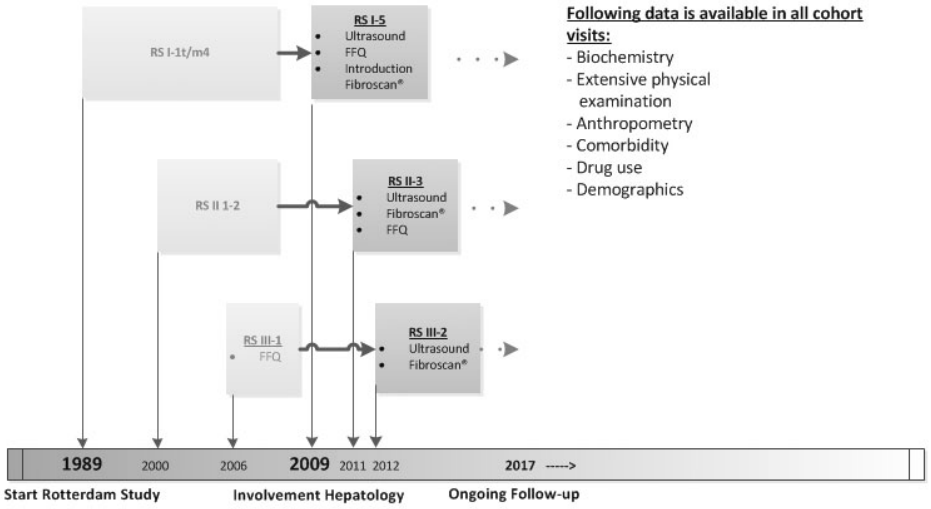
magnitude and direction of associations found in the overall analyses. However, some limitations need to be addressed. First, due to the cross-sectional design of this study, we are not able to draw conclusions regarding the cause-effect relationship of coffee, herbal tea and liver stiffness. Second, the golden standard for diagnosis of liver fibrosis remains a liver biopsy rather than TE. However, performing an invasive liver biopsy in presumed healthy participants, is unethical. TE strongly correlates with histologic stages of liver fibrosis[122] and hence, LSM appears to be a good surrogate marker for liver fibrosis in the general population.[11,78,79] Third, as with any questionnaire, data are subject to reporter- and recall-bias even though this FFQ has been extensively validated in previous studies[98,99] and unreliable FFQs were excluded. Also, none of the subjects were aware of the presence of fibrosis, since liver imaging has been conducted after dietary assessment. Fourth, unfortunately FFQs do not provide information on type of coffee or method of preparation. Therefore we could not further specify type of coffee consumption. However, previous studies have been inconclusive about whether type of coffee is of any influence. Fifth, in light of a healthy lifestyle, the association between herbal tea and liver stiffness could be affected by residual confounding even though we adjusted for a great number of lifestyle factors. Lastly, part of our study population completed the FFQs 5.8 years prior to liver imaging. Since dietary data are known to be globally stable, we assumed that drinking habits for coffee and tea did not change significantly over time in this group either.[109] This assumption is supported by our sensitivity analysis, showing that magnitude and direction of results in both groups (i.e. RS I-5 and RS II-3 vs. RS III-2) was very similar to the overall analyses (*supplementary results*).

In conclusion, we found a protective association between coffee and liver stiffness that not only occurs in disease-specific settings but appears also to be present in the general population with and without steatosis. Since coffee is an accessible and relatively inexpensive beverage, it could be further implemented as preventative strategy if future studies were to confirm our findings. Though we did not find an association with steatosis per se, coffee and tea consumption might still be useful to prevent progression to more advanced stages, such as inflammation and in particular, fibrosis. Prospective studies are needed to establish a cause-effect relation between coffee and liver fibrosis. Herbal tea consumption was also independently related to lower liver stiffness, even though consumed in small quantities. To date, very little is known about herbal tea and its effects on human health, let alone, its mechanisms of action in liver disease. Future studies are therefore needed to validate our data on the protective effect of herbal tea on liver stiffness.

Supplementary Files

Supplementary Methods: The Rotterdam Study

In short, since 1989, a total of three longitudinal cohorts (cohort RS I starting 1989; cohort RS II starting 2000, cohort RS III starting 2006) of inhabitants of Ommoord have undergone extensive home interviews and serial examinations (every 4–5 years) including, but not limited to, physical examination, laboratory assessment, cardiovascular and neurological imaging, neuropsychiatric analyses, and testing on ophthalmological, dermatological, metabolic and hepatic diseases. Liver assessments were only included into the core protocol of the Rotterdam Study from 2011 onwards. For the purpose of our study, all participants visiting the research centre between January 2011 and September 2013 (5th visit of the first cohort (RS I-5), 3rd visit of the second cohort RS II-3, and 2nd visit of the third cohort RS III-2) were included and underwent anthropometric assessment, abdominal ultrasound, transient elastography, and blood sampling. Food frequency questionnaires were available for (RS I-5, RS II-3 and RS III-1). Follow-up time between RS III-1 and III-2 is 5.8 (IQR 5.7–6.0) years.



Supplementary Figure: The Rotterdam Study Design and Timeline

Supplementary Table 1: Characteristics of low (no or moderate) vs. frequent coffee and tea consumers.

	<3 cups coffee/ day n=864	≥3cups coffee/ day n=1560	*P*-value	<3 cups tea/ day n=1738	≥3cups tea/ day n=686	*P*-value
Demographics						
Age (years)	68.2 ± 8.1	65.6 ± 6.7	<0.001	66.6 ± 7.2	66.3 ± 7.8	0.442
Female	63.0	53.0	<0.001	51.2	70.3	<0.001
Caucasian	92.8	98.2	<0.001	96.2	96.5	0.718
Low / Intermediate / High education	48.7 / 27.9 / 23.5	43.0 / 30.6 / 26.4	0.026	44.9 / 31.5 / 23.5	45.2 / 24.9 / 30.0	<0.001
Excessive alcohol	14.0	16.9	0.060	16.9	13.3	0.027
Current / Past / Never smoker	7.1 / 49.2 / 43.7	13.7 / 55.0 / 31.3	<0.001	13.3 / 53.7 / 33.0	6.3 / 50.9 / 42.7	<0.001
Physical activity*	46 (18–84)	46 (19–85)	0.540	46 (19–85)	46 (20–84)	0.852
Physical Examination						
BMI (kg/m^2)	27.0 ± 4.1	27.3 ± 3.9	0.055	27.4 ± 3.9	26.7 ± 4.1	<0.001
Normal	32.8	28.0	0.003	27.0	36.6	<0.001
Overweight	46.7	50.5		50.3	46.3	
Obese	20.5	21.5		22.7	17.2	
WC (cm)						
Men	99.7 ± 10.7	98.8 ± 9.9	0.209	99.7 ± 10.2	96.6 ± 9.7	<0.001
Women	88.4 ± 11.6	89.4 ± 11.4	0.118	89.5 ± 11.2	88.0 ± 12.0	0.022
Biochemistry						
AST (U/L)	25 (21–29)	24 (21–28)	0.007	24 (21–28)	24 (21–28)	0.268
ALT (U/L)	18 (15–23)	18 (14–24)	0.956	18 (14–24)	17 (14–23)	0.038
Bilirubin (μmol/L)	8 (7–11)	8 (6–10)	0.002	8 (6–11)	8 (6–10)	0.178
ALP (U/L)	68 (57–80)	66 (56–77)	0.011	67.7 ± 17.6	70.0 ± 17.8	0.005
GGT (U/L)	24 (16–34)	24 (17–35)	0.453	25 (18–35)	21.5 (16–32)	<0.001
Platelets (*109/L)	267.4 ± 64.1	268.0 ± 63.2	0.830	265.9 ± 64.0	272.6 ± 62.0	0.019
HOMA-IR	2.6 (1.7–4.8)	2.5 (1.7–3.8)	0.079	2.6 (1.7–4.1)	2.3 (1.6–3.4)	<0.001
Comorbidity						
Metabolic Syndrome	47.0	43.1	0.080	48.2	35.0	<0.001
- WC >88cm (♀) or >120cm (♂)	45.7	43.2	0.247	45.8	39.6	0.006
- Triglycerides >150mg/dL	41.1	36.6	0.032	40.2	32.9	0.001
- HDL-C <40mg/dL (♂) or 50mg/dL (♀)	36.7	31.9	0.019	35.9	27.8	<0.001
- Blood pressure ≥130/85mmHg¥	80.4	78.3	0.284	81.4	73.0	<0.001
- FPG >100mg/dL	45.5	44.1	0.548	47.3	37.7	<0.001

Supplementary Table 1 (*continued*)

	<3 cups coffee/ day n=864	≥3cups coffee/ day n=1560	*P*-value	<3 cups tea/ day n=1738	≥3cups tea/ day n=686	*P*-value
Diabetes Mellitus	13.2	9.1	0.002	11.3	8.6	0.054
Hypertension¥	66.5	63.7	0.213	67.5	57.6	<0.001
Liver Imaging						
LSM (kPa)	4.8 (3.8–5.9)	4.7 (3.8–5.7)	0.004	4.8 (3.8–5.9)	4. 6 (3.7–5.6)	0.008
Steatosis	35.2	34.2	0.656	36.9	28.6	<0.001

Data are presented as mean (±SD), median (IQR) or percentage. P-value is based on T-test, Wilcoxon rank sum test, Chi-square test or Fisher's exact test.
* Physical activity in metabolic equivalent task hours/week.
¥ Blood measurement data was available for 2051 participants.
Abbreviations: ALP, alkaline phosphatase; AST, aspartate transaminase.

Supplementary Table 2 A:Sensitivity analyses of RS-III cohort (n=1360) in which FFQs were completed 5.8 years previous to all other measurements, amongst them hepatological imaging.

Coffee	Odds Ratio[1]	95% Confidence Interval	P-value
Fully adjusted model*			0.122
No	1 (ref)		
Moderate (>0–3)	0.67	0.18–2.48	
Frequent (≥3)	0.37	0.11–1.27	
	β[2]	**95% Confidence Interval**	**P-value**
Fully adjusted model*			
Coffee			
No	0 (ref)		0.019
Moderate (>0–3)	0.006	–0.076 ; 0.088	
Frequent (≥3)	–0.045	–0.121 ; 0.031	
Herbal tea			
No	0 (ref)		0.018
Any	–0.046	–0.084 ; –0.008	
Green Tea			
No	0 (ref)		0.342
Any	0.019	–0.020 ; 0.058	
Black Tea			
No	0 (ref)		0.977
Any	0.001	–0.036 ; 0.037	
Total Tea			
No	0 (ref)		0.265
Moderate (>0–3)	–0.022	–0.075 ; 0.032	
Frequent (≥3)	–0.034	–0.094 ; 0.025	

Supplementary Table 2 B:Sensitivity analyses of RS-I and II cohort (n=1064) in which FFQs were completed at the same time as all other measurements.

Coffee	Odds Ratio[1]	95% Confidence Interval	P-value
Fully adjusted model*			0.110
No	1 (ref)		
Moderate (>0–3)	0.75	0.26–2.14	
Frequent (≥3)	0.43	0.15–1.23	
	β[2]	**95% Confidence Interval**	**P-value**
Fully adjusted model*			
Coffee			
No	0 (ref)		0.065
Moderate (>0–3)	–0.047	–0.130 ; 0.035	
Frequent (≥3)	–0.071	–0.150 ; 0.008	
Herbal tea			
No	0 (ref)		0.052
Any	–0.046	–0.092 ; 0.000	
Green Tea			
No	0 (ref)		0.298
Any	0.027	–0.024 ; 0.079	
Black Tea			
No	0 (ref)		0.260
Any	–0.024	–0.066 ; 0.018	
Total Tea			
No	0 (ref)		0.814
Moderate (>0–3)	–0.016	–0.070 ; –0.039	
Frequent (≥3)	0.004	–0.059 ; 0.067	

* Adjusted for tea[‡] or coffee, energy intake, BMI, gender, age, steatosis, ALT, excessive alcohol intake, current or former smoking and HOMA-IR, soda consumption, DHDI, physical activity, cream and sugar use.

[‡]All regression models either contain total tea or tea subtypes as covariate.

[1] Multivariable logistic regression models were used to determine the association between explanatory variables coffee and tea consumption with dependent variable LSM ≥8.0 kPa. No consumption is used as reference (OR=1).

[2] Multivariable linear regression models were used to determine the association between explanatory variables coffee and tea consumption with dependent variable log-transformed LSM. No consumption is used as reference (β=0).